Table 1. Effect of Curare on the Rate of ^{32}P-Orthophosphate Incorporation. Values are presented as the Specific Activity of Tissue Inorganic Phosphorus being taken as 100, and as the Mean of Three Experiments

	Specific activities relative to inorganic P of tissue	
	Control	Curarized
ATP/IP	55·3 ± 3·6	39·2 ± 3·8
ADP/IP	44·1 ± 2·9	30·4 ± 1·2
PCP/IP	73·5 ± 9·2	51·3 ± 16·1
BP/IP	814·2 ± 17·1	1,641·9 ± 52·9

ATP, ADP, PCP, BP, IP: Specific activities of adenosine triphosphate, adenosine diphosphate, phosphocreatine phosphate, blood inorganic phosphorus, tissue inorganic phosphorus, respectively.

Table 2. Concentration of Adenine Nucleotides in Control and Curarized Group

	Micro moles of adenine nucleotides per g of fresh tissue	
	Control	Curarized
AMP	0·54 ± 0·14	0·58 ± 0·14
ADP	1·02 ± 0·33	0·83 ± 0·34
ATP	4·42 ± 0·72	3·44 ± 0·34

requirement of energy would also be decreased as an example of self-regulation or feedback in a biological system. The little differences in the concentration of adenine phosphates seem to support this view. However, the marked difference in the ratio of specific activity of blood phosphate to inorganic phosphorus of tissue in curarized muscle suggests that another factor is responsible for the phosphate entry or transport. As the detailed mechanism of the efficient cellular organization for fast resynthesis and recycling of the nucleotide is still obscure, it remains to be established whether some possible changes in the membrane permeability by curare–receptor complexes have some effect on this slowing down of high energy phosphates.

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Two-dimensional Separation of Nucleic Acid Bases on Cellulose Layers

Thin-layer chromatography on cellulose layers has been shown to be an effective method for separation and quantitative determination of nucleic acid bases, nucleosides and nucleotides[1–5] which has considerable advantages over paper chromatography. Keck and Hagen[5] recently reported a one-dimensional thin-layer chromatographic separation of the four major bases of RNA and DNA. The present communication describes a two-dimensional procedure which in this laboratory has proved useful for separating more complex mixtures of nucleic acid bases.

Preparation of cellulose layers. 20 g cellulose powder *MN* 300 (Macherey and Nagel, Düren, Germany) are suspended in 120–130 ml. distilled water, homogenized in a Waring blender for 15–20 sec, and coated on degreased glass plates in the usual way, using a Stahl-type applicator[6] adjusted to a slit width of 0·5 mm (Desaga, Heidelberg, Germany). The plates are allowed to dry at room temperature for 10–15 h.

Chromatographic procedure. Chromatography is carried out in closed rectangular jars filled with solvent to a height of about 0·8 cm. It is not necessary to saturate the tank atmosphere with solvent vapours. Solvents used are: (1) methanol/concentrated hydrochloric acid/water (70 : 20 : 10, v/v), for the first dimension; (2) *n*-butanol/methanol/water/concentrated ammonium hydroxide (60 : 20 : 20 : 1, v/v), for the second dimension. The plate is dried between the two developments for 3–5 min in a current of cold air, then for 5 min in a current of hot air (60°–70° C) in order to remove hydrochloric acid as completely as possible.

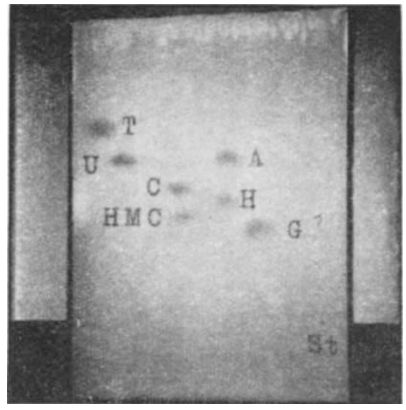

Fig. 1. Two-dimensional separation of nucleic acid bases on a 0·5 mm-cellulose layer. A solution of 0·5–1·5 μg of each compound was applied in ten 1 μl.-portions to the start spot *St*. Solvents: see text. First dimension from right to left, second dimension from bottom to top. Development distances: 10 cm in either direction. The black areas of the photograph are parts of the layer which were removed before the second development. Total development time: about 150 min. *T* = thymine; *U* = uracil; *C* = cytosine; *HMC* = 5-hydroxy-methyl cytosine; *A* = adenine; *H* = hypoxanthine; *G* = guanine. (Photography in short-wave ultra-violet light)

Fig. 1 shows a separation of a mixture of bases. The main advantages of this method are its speed, sensitivity and sharpness of resolution.

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Carbonyl Chloride Cycle in Uraemia

The role of carbonyl chloride in the production of uraemic coma has been emphasized in previous publications[1,2]. It has been further demonstrated that urea reacting with ammonium chloride *in vivo* produces carbonyl chloride with the liberation of ammonia. In view of the enormous number of cases of uraemia already handled by the author it appears that a plausible suggestion with regard to the biochemical steps involved in the production of carbonyl chloride and associated compounds is called for.

Urea is formed in the liver in the cycle described by Krebs[3], involving the mediation of arginase. In the production of uraemic coma the conversion of urea to carbonyl chloride is involved[1]. The intermediate steps may be outlined as follows:

In the first stage of the reaction one molecule of urea reacts with two molecules of free chlorine (Cl′) to produce carbonyl chloride and $2NH_2$. Further reaction with two molecules of free sodium (Na^+) results in two molecules of sodium amide in addition to the carbonyl chloride already existing. Finally, reaction with two molecules of water yields free ammonia and two molecules of sodium